



Research Article

EVALUATION OF BIOACTIVE COMPONENTS IN *ACORUS CALAMUS*

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ABSTRACT

Medicinal plants possess phytochemicals which are the main sources of medicinal drugs having curative nature and maintain vitality of individual without toxicity. The tuber part of *Acorus calamus* was collected, powdered and extracted with ethyl acetate showed an average extractive value about 200 ml. Qualitative phytochemical analysis were done and showed positive result for sterols and tannins. Quantitative estimation of tannin had relatively showed higher optical density value 0.565 than sterol 0.045 while determined through standard methods. It was confirmed that tannin, a phenolic content might be the active principle since the plant possess it as higher content. Hence, it was concluded that the plants would exploit as novel drug which drives the future that maintained the health and vitality of disease affected individual.

Keywords: Medicinal plants, *Acorus calamus*, phytochemical analysis, Tannin, Sterols

INTRODUCTION

Plants were gift of nature that has been provided essential nutritional values, medicinal properties as lifesaving antibiotics and a good source of food¹. Traditional medicinal value of herbs had opened the eyes of scientists towards scientific research explore on medicinal plants in treating or preventing the disease with prohibition of cost and side effects of allopathic treatments and to multiple drug resistant associated infections². In recent years, pharmaceutical industry made an investment to realize the potent drugs available in the market rather than new drugs.

Medicinal plants possess secondary metabolites which are the main sources of medicinal drugs having curative nature and maintain vitality of individual without toxicity³. It was believed that phytochemicals have attracted attention in recent years because of their efficacy and cost effectiveness⁴. Natural product screening was mainly dealt with the investigation of phytochemical and pharmacological approaches that eventually resulted in new drug discovery for various ailments⁵. The various parts of the plants could possess this naturally derived antibiotics and releases phytochemicals which includes steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides⁶. Qualitative screening of phytochemicals would help us to recognize what type of compounds produced by plants whereas quantitative would render us the help to know the level of quantity that was been produced.

Acorus calamus known as sweet flag referred as “Wonder drug” due to its beneficial aspect and it has been used in herbal therapies and human health care preparations for several decades. Bioactive principles in *A. calamus* possessed various pharmacological effects include antioxidant, antimicrobial, anti-diabetics, anti-

proliferative, anthelmintic, immunosuppressive and anti-carcinogenic effects⁷. Even though, many scientific reports have been suggested to use this plant against cancer but still the exact mechanism behind the anticancer property was not well understood. Considering the fact, it was strongly recommended that there was an urge for this *A. calamus* to be explored so that the chronic ailments could be totally eradicated. Thus, the focus of the current research was to investigate the phytochemicals present in the ethyl acetate extract of *A. calamus*.

MATERIAL AND METHODS

Collection of *Acorus calamus*

The tuber of *Acorus calamus* was collected from the Ayurvedic Shop, Coimbatore. The plant materials were washed thoroughly in tap water, shade dried and finely powdered. The powdered sample was stored in air tight container for further analytical purposes.

Extraction of *A. calamus*

Powdered *A. calamus* was immersed in an ethyl acetate in the ratio of 1:20 and left it for 72 h under 100 rpm. The samples were filtered using whatmann No 1 filter paper and the filtrate was concentrated at 80°C. The dried extracts were weighed and stored at 4°C. Extraction yield was calculated using the following equation⁸.

$$\text{Total Extract Yield (YT) \%} = \frac{\text{Total mass of extract}}{\text{Total mass of sample}} \times 100$$

Assessment of phytochemical presence through qualitative analysis

Test for Tannin

About 2 mg of ethyl acetate extract of *A. calamus* was treated with 5 drops of 10% ferric chloride solution and noted for the presence of bluish-black colour for tannin.

Test for Sterols and Triterpenoids

About 2 mg of ethyl acetate extract of *A. calamus* was treated with 5 drops of concentrated sulphuric acid and noted for reddish colour for the presence of sterols and yellow colour for Triterpenoids.

Test for Carbohydrates

About 2 mg of ethyl acetate extract of *A. calamus* was treated with 5 drops of Benedict's reagent (Hi-media) and boiled in water bath for the presence of orange red precipitates.

Test for glycoside

About 2 mg of ethyl acetate extract of *A. calamus* was treated with 5 drops of 10% ferric chloride and immersed in boiling water for 5 minutes and cooled. Then, equal volume of benzene was added followed by 5 drops of ammonia solution and noted for the formation of rose-pink colour in ammonia layer for glycosides

Test for Saponin

A dry weight of 2 gm of plant extract powder were diluted with distilled water to 20 ml and shaken for 15 mins and noted for the formation of 1 cm layer of foam indicates the presence⁹.

Quantitative analysis on phytochemical constituents

Estimation of Total Sterol Content (TSC)

A dry weight of 100 mg of plant extract was added with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 hours and cooled at room temperature. Solid sodium carbonate was added until effervescence ceases. It was then centrifuged and the supernatant was collected and made up to 100 ml with the help of

distilled water. A volume of 0.2 ml of diluted supernatant was taken out and added with 1 ml of distilled water followed by addition of phenol reagent subsequently with 5 ml of sulphuric acid. Then, the tubes were kept at 30°C for 20 minutes and the absorbance was taken at 490 nm¹⁰.

Estimation of Total Tannin Content (TTC)

Tannin content of plant extract was estimated by Folin-Denis method. About 50 µl of extract (mg/ml) was made up to 7.5 ml by adding distilled water. 0.5 ml Folin-Denis reagent and 1 ml of sodium carbonate was added and made up to 10 ml using distilled water. The color development was measured spectrophotometrically at 700 nm. Tannic acid (20 -120 µg/ml) was used as standard to plot curve. The results were given as µg of Tannic acid equivalents (TAE)/mg of extract¹⁰.

Estimation of Total Phenol Content (TPC)

According to Folin-Ciocalteu's method, the total phenolic content in the ethyl acetate extract of plant was determined. About 50 µl of extract (mg/ml) was made up to 7.5 ml by adding distilled water. 0.5 ml Folin-phenol reagent and incubated for 3 mins. After incubation, 1 ml of saturated sodium carbonate was added to the reaction mixture and made the volume up to 10 ml using distilled water and again incubated the solution in the dark for 90 minutes. The color development was measured spectrophotometrically at 725 nm. A standard curve was plotted with various concentrations of Gallic acid (20-100 µg/ml) standard. Final results were given as µg of Gallic acid equivalents (GAE)/mg of extract basis¹⁰.

RESULT AND DISCUSSION

Extraction step was an initial step prior to analysing the bioactive principles of herb. The collected *A. calamus* tuber was powdered and subjected for ethyl acetate extraction depicted in Figure 1 (a, b, c). Appropriate solvent selected that would impart its significance in the extract yield. Here, ethyl acetate solvent was used because it enhances the antimicrobial profiling of the plant in addition it also minimizes the toxicity grade of the selected medicinal herb since it was being used to treat chronic ailments. Extract yield was calculated and showed the average extractive value of 200 ml. This result indicated a wide range of extraction yield for ethyl acetate as showed in Figure 1 (d and e).



Figure 1a: *Acorus calamus* tuber



Figure 1b: Powdered tuber of *Acorus calamus*

Figure 1: Collection and extraction of *A. calamus*

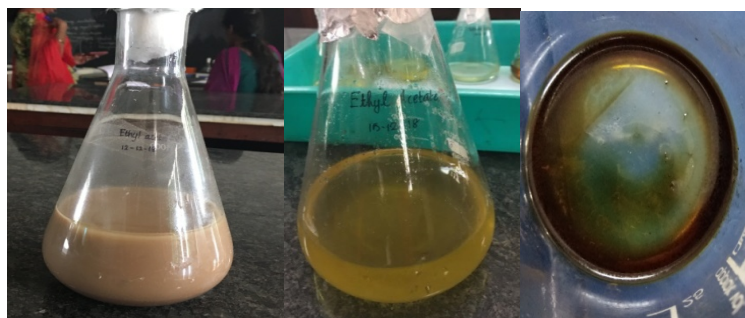


Figure 1c: Ethyl acetate extraction 1d: Filtrate 1e: Macerated extract

Table 1: *Acorus calamus* extraction

Plant name	Parts used in extraction	Method of extraction	Solvents	Colour and consistency	Average extractive value
<i>Acorus calamus</i>	Root	Cold maceration	Ethyl acetate	Brownish and turbid	200 ml

Phytochemicals were produced by the various parts of the plant such as bark, leaves, tuber, seed etc., and it was identified through qualitative tests. The ethyl acetate extract of *A. calamus* was subjected for various standard tests to identify the presence of phytochemicals. It showed positive result for sterols and tannins. According to Santhi and her co-works¹¹ statement, qualitative phytochemical screening helps us to understand a variety of chemical compounds produced by the plants and quantification of those metabolites would help us to extract, purify and identify

the bioactive compounds for useful aspects to human beings. Table 2 and Figure 2 revealed the presence of bioactive compounds, tannin and sterol whereas it showed carbohydrate, glycoside, saponin and terpenoids were absent. According to the statement rendered by Geissman¹², plants had limitless ability to synthesize aromatic substances preferably oxygen substituted derivatives like phenols because it would serve as plant defense mechanisms. However, the plant had passed successfully in the tests of commercial screenings.

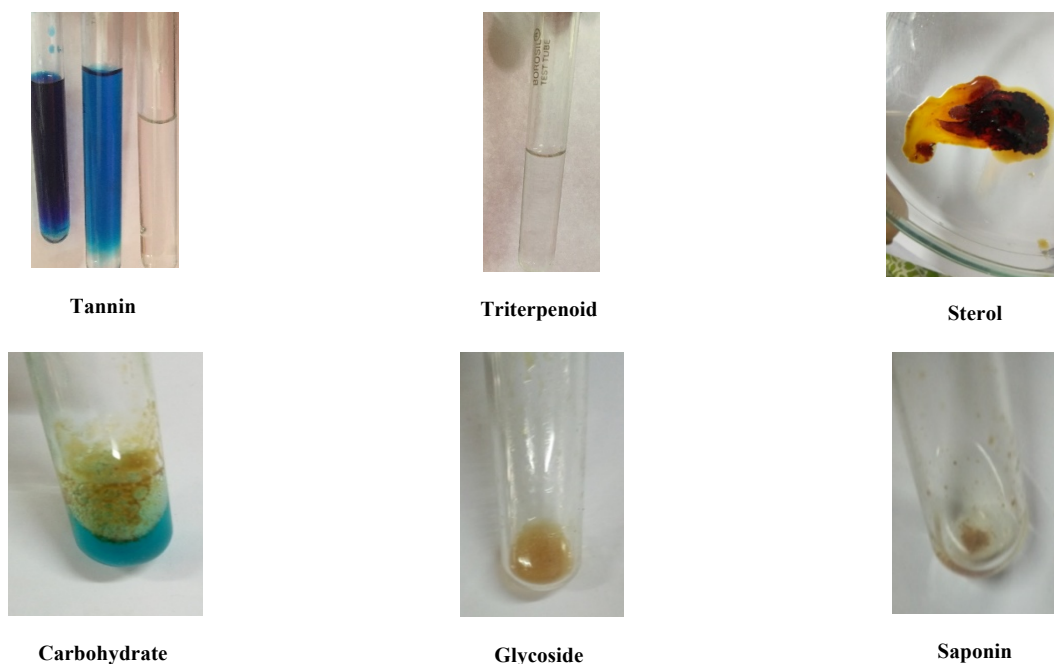


Figure 2: Qualitative phytochemical analysis of *A. calamus*

Table 2: Results for qualitative phytochemical analysis of *A. Calamus*

Phytoconstituent	Ethyl acetate
Tannin	Positive
Sterols	Positive
Triterpenoids	Negative
Glycoside	Negative
Carbohydrate	Negative
Phenol	Positive
Saponin	Negative

Quantification of screened secondary metabolites through qualitative analysis was done to quantify it. Quantitative estimation of tannin had relatively showed higher optical density value than sterol while determined through spectrophotometric methods in ethyl acetate extract of *A. calamus* tuber. Result is tabulated in Table 3.

Table 3: Quantitative analysis of screened phytochemicals

Phytochemical	Optical density
Tannin	0.565
Sterol	0.045

The present investigation showed significant variation in the contents like tannin and sterols. These variations were occurred due to various environmental factors such as climate, altitude and rain fall etc as mentioned by Kokate and co-workers¹³

CONCLUSION

Phyto constituents present in the ethyl acetate extract of *Acorus calamus* was evaluated and result showed that the tannin, a phenolic compound seemed to be bioactive compound. Raw medicinal plant part has been used nowadays for preparing therapeutic agents for chronic ailments. Based on the above-mentioned findings, it was concluded that *Acorus calamus* would play a significant role in medicinal drugs and pharmaceutical industry especially in drug formulations due to presence of important phytochemicals.

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